Journal of Food Protection 86 (2023) 100074

Contents lists available at ScienceDirect

Journal of Food Protection

journal homepage: www.elsevier.com/locate/jfp

Research Paper

Foodborne Outbreak of Extended Spectrum Beta-lactamase Producing *Shigella sonnei* Associated with Contaminated Spring Onions in the United Kingdom

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ARTICLE INFO

Keywords:
Extended spectrum beta-lactamase
Food of nonanimal origin
Foodborne shigellosis
Shigella sonnei

ABSTRACT

Globalization of the food supply chain has created conditions favorable for emergence and spread of multidrug-resistant (MDR) foodborne pathogens. In November 2021, the UK Health Security Agency detected an outbreak of 17 cases infected with the same strain of MDR extended spectrum beta-lactamase (ESBL)-producing *Shigella sonnei.* Phylogenetic analysis of whole-genome sequencing data revealed the outbreak was closely related to strains of *S. sonnei* isolated from travelers returning to the UK from Egypt. None of the outbreak cases reported travel and all 17 cases reported eating food from a restaurant/food outlet in the week prior to symptom onset, of which 11/17 (64.7%) ate at branches of the same national restaurant franchise. All 17 cases were adults and 14/17 (82.4%) were female. Ingredient-level analyses of the meals consumed by the cases identified spring onions as the common ingredient. Food chain investigations revealed that the spring onions served at the implicated restaurants could be traced back to a single Egyptian producer. The foodborne transmission of ESBL-producing bacteria is an emerging global health concern, and concerted action from all stakeholders is required to ensure an effective response to mitigate the risks to public health.

Surveillance of foodborne pathogens, including the detection and investigation of outbreaks of foodborne gastrointestinal disease in England, falls under the remit of the UK Health Security Agency (UKHSA) (https://www.gov.uk/government/collections/gastrointestinal-infections-guidance-data-and-analysis), in collaboration with colleagues from the Food Standards Agency (FSA) (https://www.food.gov.uk), and local authority environmental health teams. Whole-genome sequencing (WGS) contributes to the surveillance strategies for *Listeria monocytogenes,* Shiga toxin-producing *Escherichia coli* (STEC), *Salmonella, Campylobacter,* and *Shigella* species (Ashton et al., 2016; Chattaway et al., 2017; Jenkins et al., 2019; Painset et al., 2019; Tewolde et al., 2016). Although *Salmonella* and *Campylobacter* species contribute to the highest burden of gastrointestinal infections in the UK (https://www.gov.uk/government/publications/salmonella-national-laboratory-and-outbreak-data; https://www.gov.uk/government/publications/campylobacter-infection-annual-data/campylobacter-data-2008-to-2017), *L. monocytogenes,* STEC, and *Shigella* species are also important because infections are more likely to be associated with severe clinical outcomes (https://www.gov.uk/government/publications/listeria-monocytogenes-surveillance-reports, Bardsley et al., 2020; Butt et al., 2022). Furthermore, a high proportion of *Shigella* species are multidrug-resistant (MDR) and treatment options are often limited (Charles et al., 2022; Urban-Chmiel et al., 2022).

Unlike the majority of gastrointestinal pathogens under surveillance at UKHSA, the shigellae are not zoonotic and in the UK, transmission usually occurs via person-to-person contact. However, transmission via the foodborne route can occur if food or water become contaminated with human feces and/or are prepared by an infected food handler (Jenkins, 2016). Foodborne outbreaks of shigellosis in the UK and elsewhere have been associated with ready-to-eat food items, including salad vegetables and fresh herbs, contaminated by human feces at source or subsequently by infected food handlers

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https://doi.org/10.1016/j.jfp.2023.100074
Received 21 December 2022; Accepted 10 March 2023
Available online 7 April 2023

(Frost et al., 1995; Lewis et al., 2009; McLarty et al., 2022; Mikhail et al., 2021; Muller et al., 2009).

Shigellosis, or bacillary dysentery, is characterized by a rapid onset of profuse, watery and/or bloody diarrhea, abdominal pain, and sometimes fever. Treatment is often recommended in patients when symptoms are severe and/or prolonged, and/or the patient is very old or very young. There are four species of *Shigella* as follows: *S. sonnei, S. flexneri*, *S. boydii,* and *S. dysenteriae*. *S. boydii* and *S. dysenteriae* are rarely detected in UK residents and are almost exclusively linked to travelers' diarrhea (Terry et al., 2018; Todkill et al., 2018). *S. sonnei* and *S. flexneri* are commonly reported and are associated with sexual transmission among gay, bisexual, and other men who have sex with men, travelers' diarrhea, community outbreaks often linked to schools, as well as outbreaks of foodborne disease (Bardsley et al., 2020; McLarty et al., 2022; Mikhail et al., 2021; Rew et al., 2018).

On 16 November 2021, the UKHSA Health Protection Team (HPT) in the South West region of England initiated an investigation into a cluster of five cases of *S. sonnei*. Initial epidemiological investigations revealed that cases reported eating out at the same national restaurant franchise. Case ascertainment using WGS typing data identified additional cases resident in two other regions of England . In order to consolidate and coordinate information and communications, the incident was escalated to a national response on 26 November 2021. Here, we describe the epidemiological and microbiological investigation of the outbreak, assess the risk to public health from foodborne MDR *S. sonnei*, and make recommendations for future practice.

Materials and Methods

Clinical investigations. Fecal samples were processed at local hospital laboratories for routine microbiological testing for enteric pathogens (https://www.gov.uk/government/publications/smi-b-30-investigation-of-faecal-specimens-for-enteric-pathogens). Isolates found to be positive for *Shigella* species were submitted to the Gastrointestinal Bacterial Reference Unit (GBRU) at the UKHSA for confirmation of species identification and typing, in concordance with local practice (Bardsley et al., 2020). Since 2015, species identification and molecular typing have been performed using WGS (Chattaway et al., 2017; Dallman et al., 2016, Sadouki et al., 2015).

Epidemiological investigations. Cases were defined as follows:

Confirmed case: an individual with *S. sonnei* confirmed at the GBRU that fell within 5-single nucleotide polymorphism (SNP) single linkage cluster with the UKHSA SNP type designated t5.2559.

Probable case: an individual who had laboratory-confirmed *S. sonnei* isolated from a stool specimen dated 25th October 2021 or later; and consumed food or drink prepared by a restaurant or food outlet (including takeaway) within 7 days before symptoms onset; and did not have a whole-genome sequencing result available.

Diagnoses of shigellosis are notified to local HTPs via the second generation surveillance system (SGSS), an application that captures routine laboratory surveillance data on infectious diseases from diagnostic laboratories across England (https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/926838/PHE_Laboratory_reporting_guidelines_October-2020-v3.pdf). Following this initial notification, questionnaires are administered by environmental health practitioners (EHPs) to all cases of *S. flexneri, S. boydii,* and *S. dysentaeriae*, and cases of *S. sonnei* where there is a risk of foodborne transmission (for example, if the case is a food handler) or if a foodborne outbreak is suspected (https://www.gov.uk/government/publications/shigellosis-public-health-management-and-questionnaire). Data captured includes demographic information (age, sex, and occupation), symptom information (including onset, duration, and severity), national and international travel and food histories during the 7 days prior to onset of symptoms.

Case-control study. A retrospective case-control study was conducted. The study population were individuals who consumed food or drink from a restaurant or food outlet (including takeaway), identified by at least one case, between 25 October and 11 November 2021 and who were exposed at the same time and place as a case (see case definitions below). Case data were collected from the surveillance questionnaires completed as part of routine follow-up following a notification of shigellosis where foodborne transmission or outbreak is suspected. Cases were then re-contacted to identify co-diners as controls, and to confirm the information they provided. Cases were asked to share a Select Survey questionnaire with their co-diners, which captured basic demographics and exposure history through the identification of meal items consumed while codining with cases. Local authority environmental health officered then contacted the identified restaurants to request lists of ingredients for food and drink items consumed by cases and controls.

Due to multiple shared ingredients between dishes, the food and drink exposure variables for analytical epidemiology were at ingredient level rather than dishes or drinks consumed. In the univariate analyses, the ingredient exposures of cases and controls were compared, and unadjusted odds ratios (OR) and 95% confidence intervals (CIs) were calculated. Due to small sample size, p values were obtained by using Fisher's exact test in an analysis assuming independence. Where there were sampling zeros, Firth regression was used to estimate ORs and p values were obtained using the Wald test. All ingredients with an OR $>$ 1 and a p value of <0.4 in the univariate analyses were considered for inclusion in the multivariable models. The ingredient with the highest odds ratio in the univariate analyses was the primary ingredient of interest. Multivariable Firth logistic regression analyses were conducted which included the primary ingredient of interest and each of the other ingredients of interest. Models were limited to including only two explanatory variables due to the small sample size and the risk of overfitting the model. The change in the unadjusted OR to the adjusted OR in each of the models indicated if the other ingredients of interest potentially confounded the relationship between the consumption of the primary ingredient of interest and the odds of being a case. Goodness of fit tests were not calculated given the small sample size. R version 4.1.0 (2021-05-18) (RStudio Desktop version 2021.09.1 + 372) was used for data cleaning and analysis. Packages used included Tidyverse, gtsummary, and logistf packages.

Case-control study case definitions

Confirmed case: an individual with *S. sonnei* confirmed at the GBRU that fell within 5-single nucleotide polymorphism (SNP) single linkage cluster with the UKHSA SNP type designated t5.2559.

Probable case: an individual who had laboratory-confirmed *S. sonnei* isolated from a stool specimen dated 25th October 2021 or later; and consumed food or drink prepared by a restaurant or food outlet (including takeaway) within 7 days before symptoms onset; and does not have a whole-genome sequencing result available.

Exclusion criteria for confirmed and probable cases: an individual who reported international travel in the 7 days prior to the onset of symptoms are likely to have acquired *S. sonnei* infection from a household member or close contact (i.e. the case had a household member with onset date in the 7 days before the case's onset date).

Any restaurants or food outlets visited within 7 days post symptom onset of laboratory-confirmed *S. sonnei* infection were discounted as a possible exposure.

Controls: an individual who consumed food or drink from a restaurant or food outlet (including takeaway) identified by at least one case since 25th October 2021 and who was exposed at the same time and place as a case; and did not have a clinically compatible history of shigellosis or laboratory-confirmed *S. sonnei* or untyped *Shigella* infection since 25th October 2021

Analysis of sequencing data. Genomic DNA from isolates of *S. sonnei* routinely submitted to GBRU, UKHSA, was extracted for sequencing on the Illumina NextSeq 1000 instruments. Read trimming and multilocus sequence typing were performed as previously described (Dallman et al., 2016). High-quality Illumina reads were mapped to the *S. sonnei* reference genome Ss46 (Genbank accession: NC_007384.1) (Wei et al., 2003) using BWA MEM v0.7.12 and Samtools v1.1 (Li & Durbin, 2010; Li et al., 2009). Single Nucleotide Polymorphisms (SNPs) were identified using GATK v2.6.5 in unified genotyper mode (McKenna et al., 2010). Core genome positions that had a high-quality SNP (>90% consensus, minimum depth 10×, GQ >= 30) in at least one isolate were extracted and IQtree v2.0.4 was used to derive the maximum likelihood phylogeny of the isolates after first removing regions of the genome predicted to have undergone horizontal exchange using Gubbins v2.0 (Croucher et al., 2015; Dallman et al., 2018; Stamatakis, 2014). SNP typing data, in the form of SNP address, are linked to patient data and stored in a web-based database called the Gastro Data Warehouse. A WGS cluster is defined as two or more cases for which the sequences of the isolates are within 0, 5, or 10 SNPs of each other (Dallman et al., 2018).

Resistance determinants are often encoded on mobile genetic elements (MGEs), such as plasmids. Their location and architecture can be challenging to investigate using short-read sequencing data due to difficulties with the assembly of repetitive and paralogous regions that are characteristic of MGEs. Long-read sequencing data were generated using the Nanopore sequencing platform for 14 strains to characterize and determine the genomic locus of key resistance determinants, such as $bla_{\text{CTX-M}}$ variants, as described previously (Charles et al., 2022; Greig et al., 2021). All FASTQ and assemblies were submitted to the National Centre for Biotechnology Information (NCBI). Illumina FASTQ accession numbers are listed in Figure 5 and the supplementary table. Nanopore FASTQ accession numbers are listed in the supplementary table. All FASTQ data are publicly available in BioProject: PRJNA315192.

Food and environmental investigations. EHPs collected information on food items served at the restaurants and food outlets linked to the outbreak, including the details of each ingredient used per dish. Forward and backward food chain information was collected for all ingredients of meals eaten by cases at the venues.

All food specimens collected as part of this outbreak investigation were examined using an in-house method. Testing was conducted at the UKHSA Food, Water, and Environment (FW&E) laboratory in Porton. A 25 g portion of each food sample was homogenized with sufficient Buffered Peptone Water (BPW) supplemented with novobiocin (0.5 μg/mL), to achieve a 10^{-1} dilution; environmental swabs were submerged in 100 mL of BPW. Homogenates were incubated at 41.5°C for 18 h, and 10 μL aliquots were then subcultured to MacConkey and Xylose Lysine Desoxycholate (XLD) agar plates. Plates were incubated at 37°C for 22 h, and suspect colonies were subjected to confirmatory testing (oxidase test and agglutination with antiserum specific to *S. sonnei*).

In addition, a real-time polymerase chain reaction (PCR) technique was used to examine samples for the presence of *Shigella* species. The real-time multiplex PCR assay was performed using the TaqMan Environmental Mastermix 2.0 on a Taqman 7500 instrument (Applied Biosystems). The reaction mixture consisted of 12.5 μL TaqMan Environmental Mastermix (polymerase, PCR buffer, deoxynucleoside triphosphate mix, and $MgCl_2$), 10 μM concentrations of TQipaHfwd (TGCATGGCTGGAAAAACTCA) and TQipaHrev (CAGCAGCAACAGCGAAAGACT) primers, and TQ *ipaH* probe (FAM CTGCGGAGCTTCCA MGB), TaqMan™ Exogenous Internal Positive Control (IPC) Reagents (4308323 Applied Biosystems) (2.5 μL of 10 x EXO IPC Mix and 0.5 μL 50 x EXO IPC DNA), 0.25 μL of AmpEase Uracil N-Glycosylase (UNG), 5 μL of sample template, and PCR-grade water to a final volume of 25 μL. Negative controls containing PCR-grade water for sample template and a positive control with boiled lysate dilutions from control strain *S. sonnei* (Ss46) were included in every run. Cycling conditions began with a 2 min hold at 50°C to activate the UNG followed by 10 min at 95°C to denature and then followed by 40 cycles of 15 s at 95°C and 1 min at 60°C.

Results

Descriptive epidemiology. There were 17 confirmed cases of *S. sonnei* t5.2559 identified with sample dates between 5th and 24th December 2021 and two probable cases (Fig. 1), with onset dates from 2nd November to 16th December. Most confirmed cases were female 82.4% (14/17), and ages ranged from 18 years to 63 years (median = 29 years) (Fig. 2). The majority of confirmed cases (76.5%, 13/17) lived in the South West of England. Of the remaining four confirmed cases, one was resident in the South East and three in London (Fig. 3).

Exposure information was gathered through surveillance questionnaires for shigellosis. A total of 17/19 (88%) of cases reported eating out at, or ate a takeaway from, a restaurant/food outlet in the 7 days prior to symptom onset. Of these, 11/17 (64.7%) of cases consumed food from a national restaurant chain, Restaurant Z (Branch A, n = 9; Branch B, n = 1; Branch C, n = 1). Ingredient-level analyses of the meals consumed by the cases identified spring onions as the common ingredient (Table 1). Six cases had no known links to Restaurant Z, however, four of these reported additional restaurant exposures of which two cases recalled consumption of spring onions. Two cases did not report eating out or takeaway in the week prior to onset of symptoms.

Analytical case-control study. A total of 13 individuals met the case definition and six met the control definition. Symptom onset dates for the cases ranged from 2nd November to 6th November. The median age of cases was 30 years (range 17–62) and 31 years for controls (range 18–38). The majority of cases and controls reported eating at Branch A of Restaurant Z. Spring onion had associated increase in odds of being a case (OR 15, 95% CI 0.60, 375) (Table 2). The multivariable is the strongest evidence of association with illness at the univariable level (OR 15, 95% CI 0.60, 375, p = 0.1) (Table 3). The analyses also indicated that firecracker sauce, sweet potato, sticky rice, cucumber, and kimchee were potential confounders, indicated by the change in OR for spring onions, however, the spring onions still retained a degree of significance (p value <0.2). Given the low number of controls, a p value of less than or equal to 0.2 was used to indicate evidence of association with illness.

Food and environmental sampling and food chain investigations. In total, 65 samples (14 environmental swabs and 51 foods) were examined from two of the branch premises of Restaurant Z. All tests on these samples gave negative results for *Shigella* species, including three samples of spring onions.

Suppliers to Restaurant Z were established as Supplier 1 for fresh produce, including spring onions, and Supplier 2 for ambient and frozen foods. Spring onions used by Restaurant X were also provided by Supplier 1. Food origin information from Supplier 1 indicated that spring onions were sourced from the UK in October; however, they changed to an Egyptian grower (Producer 1) in November. Supplier 1 received produce from Supplier 3, who also sourced from Producer 1 in Egypt. The suppliers to Restaurant Y were established as Supplier 4, who also sourced spring onions from Supplier 3. Ultimately, the food chain investigation showed that three of the implicated restaurants purchased spring onions from two different supply chains, and that both supply chains were supplied by the same Egyptian grower (Producer 1) (Fig. 4).

A notification to the EU of the investigation in the UK was distributed through International Health Regulation on 2 December 2021. Six EU countries responded to confirm they had not identified any increases in *S. sonnei* notification, nor had they detected any

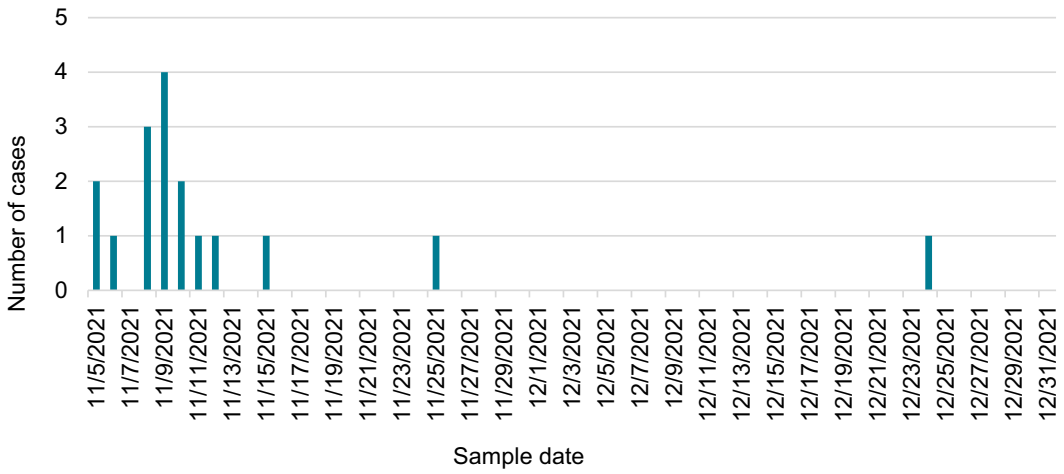

Figure 1. Number of confirmed cases of *S. sonnei* t5.2559 by sample date (n = 17).

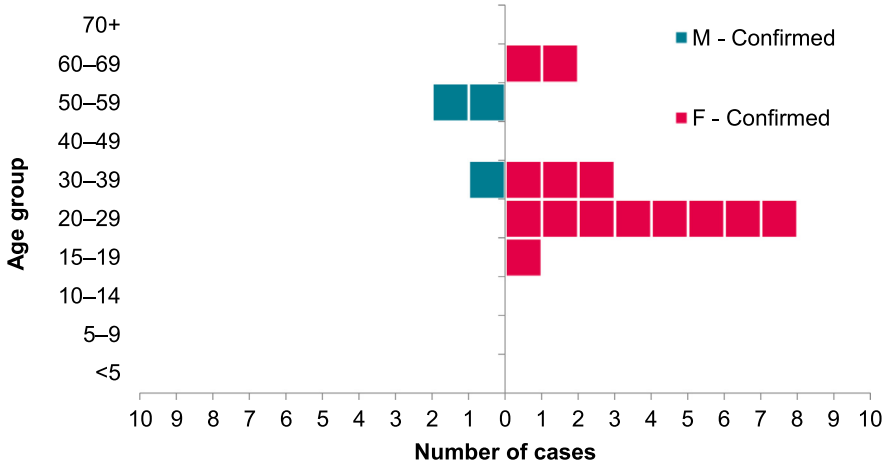

Figure 2. Age and sex distribution of the confirmed cases of *S. sonnei* t5.2559 (n = 17).

isolates of *S. sonnei* that belonged to same 5-SNP single linkage cluster as the outbreak strain. The FSA notified their international food safety counterparts via the International Food Safety Authorities Network (INFOSAN) Emergency Focal Points.

Microbiology and genomic investigations. The isolates falling within the t5:2559 cluster were genetically closely related, exhibiting a maximum SNP distance of eight SNPs across the entire t5 cluster, and an average of one SNP difference between isolates, indicating association with a common source of contamination (Fig. 5). The outbreak strain was located 22–25 SNPs distant from its nearest neighbor. There were isolates in the wider phylogeny within 25 SNPs of the outbreak strain, from cases reporting recent travel to Egypt (Fig. 5).

The outbreak strain was MDR and had genetic markers predicted to confer reduced susceptibility to third-generation cephalosporins (ESBL-producer harboring $bla_{\text{CTX-M-15}}$), aminoglycosides (*strA-strB*, *addA1*), fluroquinolones (*qnrS1*), trimethoprim (*dfrA1*), tetracycline (*tetA-1),* and sulphonamides (*sul2*). Analysis of long-read sequencing data confirmed that $bla_{\text{CTX-M-15}}$ and *qnrS1* were located on a 77kbp IncFII plasmid (Fig. 6).

Discussion

Foodborne outbreaks of shigellosis, although rare in the UK, are a public health concern because they may be associated with severe clinical outcomes and the causative agent is often MDR (McLarty et al., 2022; Mikhail et al., 2021). Gastrointestinal infections are usually self-limiting; however, antibiotics are recommended when symptoms are severe or prolonged, or if the patient is at the extreme ages of life or has an underlying health condition (https://www.gov.uk/government/publications/shigellosis-public-health-management-and-questionnaire). In this study, the outbreak strain exhibited reduced susceptibility to fluroquinolones and third-generation cephalosporins, the recommended first and second-line treatment options for shigellosis in the UK, respectively. Analysis of the nanopore sequencing data confirmed that *qnrS1* and $bla_{\text{CTX-M-15}}$ genes were located on the same IncFII plasmid, highlighting the risk of *in vivo* transmission of this mobile genetic element to other bacteria in the gut. Acquisition of plasmids encoding $bla_{\text{CTX-M}}$ variants by commensal bacteria colonizing the host gastrointestinal tract may result in persistence and long-term shedding of bacteria resistant to the third-generation cephalosporins, often referred to as the “hospital workhorse” antibiotics (Arcilla et al., 2016; Bevan et al., 2018; Klein & Cunha, 1995).

There are a number of reports in the literature of outbreaks of *S. sonnei* caused by leafy greens, including fresh herbs, and salad vegetables (Frost et al., 1995; Lewis et al., 2009; McLarty et al., 2022; Mikhail et al., 2021; Muller et al., 2009). The shigellae are known to have a low infectious dose, and there is evidence that washing these items does not remove all the bacterial contamination (Jenkins, 2016). Foodborne outbreaks caused by contaminated salad vegetables are challenging, and such products have been referred to as ‘stealth

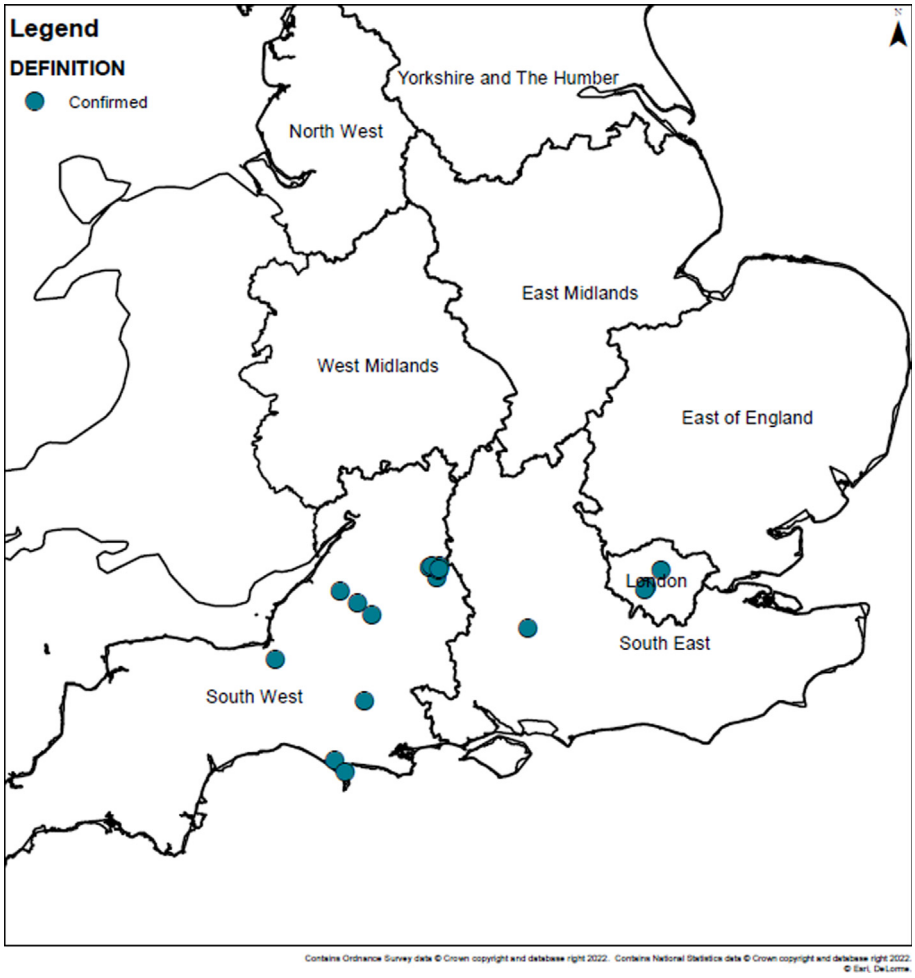

Figure 3. Geographical distribution of the confirmed cases of *S. sonnei* t5.2559 (n = 17).

Table 1
Ingredient analyses derived from meals consumed by cases linked to Restaurant Z (n = 11)

Ingredient	Number
spring onion	11 (100%)
Rice	9 (82%)
Kimchi	8 (73%)
rapeseed oil	7 (64%)
sesame seeds	7 (64%)
Carrots	6 (55%)
Chicken	6 (55%)
pea shoots	6 (55%)
yakitori sauce	6 (55%)
Cucumber	5 (45%)
Ginger	5 (45%)
red onion	5 (45%)
cooked hirata buns	4 (36%)
edamame beans	4 (36%)
teriyaki sauce	4 (36%)

vehicles', as cases often fail to recall consumption of items served as a side-dish or as a minor ingredient of the main meal (https://www.barf-blog.com/2012/10/stealth-ingredient-e-coli-threat-prompts-eu-sprouted-seeds-measures/, Byrne et al., 2016; Kintz et al., 2019). Furthermore, salad vegetables have a short shelf life and by the time a potential vehicle has been identified and microbiological sampling has commenced, the contaminated batch is no longer available for testing (Kintz et al., 2019).

In this outbreak investigation, most cases had eaten out at a restaurant in the days prior to symptom onset and were able to recall the meal they had ordered. It was then possible to identify the specific ingredients for each meal and cross-check for common items (Gardiner et al., 2018). This preliminary analysis identified spring onions as a potential vehicle. The comprehensive and detailed trace-back investigation conducted by the FSA revealed that three of the implicated restaurants purchased spring onions from two different supply chains, and that both supply chains sourced spring onions from the same grower in Egypt. Further evidence of the link to Egypt was the geographical signal observed from analyzing travel histories of cases infected with strains of *S. sonnei* phylogenetically closely related to the outbreak strain. The results from the analysis of the epidemiological and genomic data, and the outcome of the trace-back investigation, were deemed sufficient evidence that contaminated spring onions from Producer 1 were the contaminated vehicle, despite the absence of microbiological evidence from the food and environmental sampling.

Although the outbreak was initially detected by the local HPT following the analysis of the cases questionnaire data and the identification of the link to Restaurant Z, additional cases were ascertained using the sequencing data and were identified because these cases were infected with isolates of *S. sonnei* that fell within a 5-SNP single linkage cluster of the outbreak strain (Dallman et al., 2016, 2018). Genome sequences from foodborne pathogens generated at UKHSA are routinely made publicly available in real-time, and during this outbreak, the sequences were shared with public health colleagues globally. No matches with isolates of *S. sonnei* in other countries were

Table 2
Univariate analyses

Ingredient	Consumed			Not consumed			Odds Ratio	95% CI[a]	p value
	Cases	Noncases	Odds	Cases	Noncases	Odds			
spring onion	13	4	3.25	0	2	0	15.0	0.60, 375	0.10[b]
firecracker sauce	3	0	-	10	6	1.67	4.3	0.19, 98	0.36[b]
sweet potato	3	0	-	10	6	1.67	4.3	0.19, 98	0.36[b]
sticky rice	6	1	6	7	5	1.4	4.0	0.31, 235	0.33[c]
cucumber	6	1	6	7	5	1.4	4.0	0.31, 235	0.33[c]
Kimchee	6	1	6	7	5	1.4	4.0	0.31, 235	0.33[c]
yakitori sauce	6	1	6	7	5	1.4	4.0	0.31, 235	0.33[c]

[a] CI = Confidence Interval.
[b] Univariate Firth regression (p values obtained by the Wald test).
[c] Fisher's exact t.

Table 3
Multivariable analysis using Firth logistic regression

Ingredients	Odds Ratio	95% CI[1]	p value
spring onion	11.7	0.46, 295	0.14
firecracker sauce	3.00	0.13, 70.9	0.5
[a]CI = Confidence Interval			
Ingredients	Odds Ratio	95% CI[1]	p value
spring onion	11.7	0.46, 295	0.14
sweet potato	3.00	0.13, 70.9	0.5
[a]CI = Confidence Interval			
Ingredients	Odds Ratio	95% CI[1]	p value
spring onion	10.7	0.40, 288	0.2
sticky rice	2.02	0.23, 17.9	0.5
[a]CI = Confidence Interval			
Ingredients	Odds Ratio	95% CI[1]	p value
spring onion	10.7	0.40, 288	0.2
Cucumber	2.02	0.23, 17.9	0.5
[a]CI = Confidence Interval			
Ingredients	Odds Ratio	95% CI[1]	p value
spring onion	10.7	0.40, 288	0.2
Kimchee	2.02	0.23, 17.9	0.5
[a]CI = Confidence Interval			
Ingredients	Odds Ratio	95% CI[1]	p value
spring onion	10.7	0.40, 288	0.2
yakitori sauce	2.02	0.23, 17.9	0.5
[a]CI = Confidence Interval			

identified; however, colleagues at the Statens Serum Institute in Denmark reported a concurrent outbreak of enteroinvasive *Escherichia coli* (EIEC) linked to spring onions from the same grower in Egypt, Producer 1 (Personal communication: Mia Torpdahl & Susanne Schjørring, Statens Serum Institute, Copenhagen, and Torpdahl et al., 2023). Danish colleagues reported that at harvest time, there was evidence of a flooding event and they concluded that human fecal contamination in the river water had contaminated the crop. It is plausible that this event contributed to the temporally linked outbreak described in this study, despite the two outbreaks being caused by different pathogens. Flooding events have been identified as the cause of a number of outbreaks of gastrointestinal infections (Charron et al., 2004; Kintz et al., 2019; Na et al., 2016).

The foodborne transmission of multidrug-resistant bacteria is an emerging clinical and public health concern (Alikhan et al., 2022; Davies et al., 2022; Larkin et al., 2022; Mikhail et al., 2021; Urban-Chmiel et al., 2022). Globalization of the food supply has created conditions favorable for emergence, reemergence, and spread of MDR foodborne pathogens (https://nap.nationalacademies.org/read/13423/chapter/2#77). In this study, the epidemiological data from case questionnaires, including food histories and travel histories, were integrated with WGS data, and used to direct the investigation and inform the food chain trace-back investigation. This ultimately led to the identification of the vehicle of infection. Coordinated action from

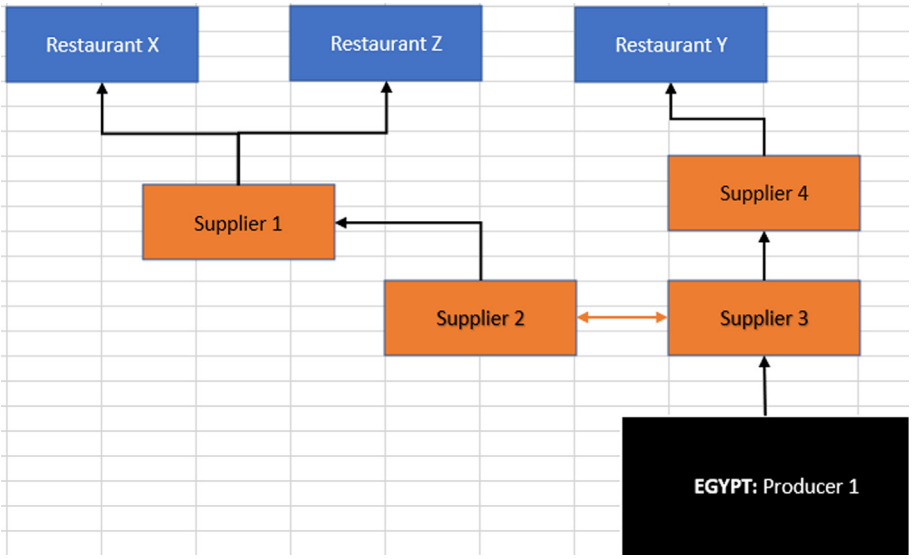

Figure 4. Food chain supply lines showing that three of the implicated restaurants sourced spring onions from the same Egyptian producer.

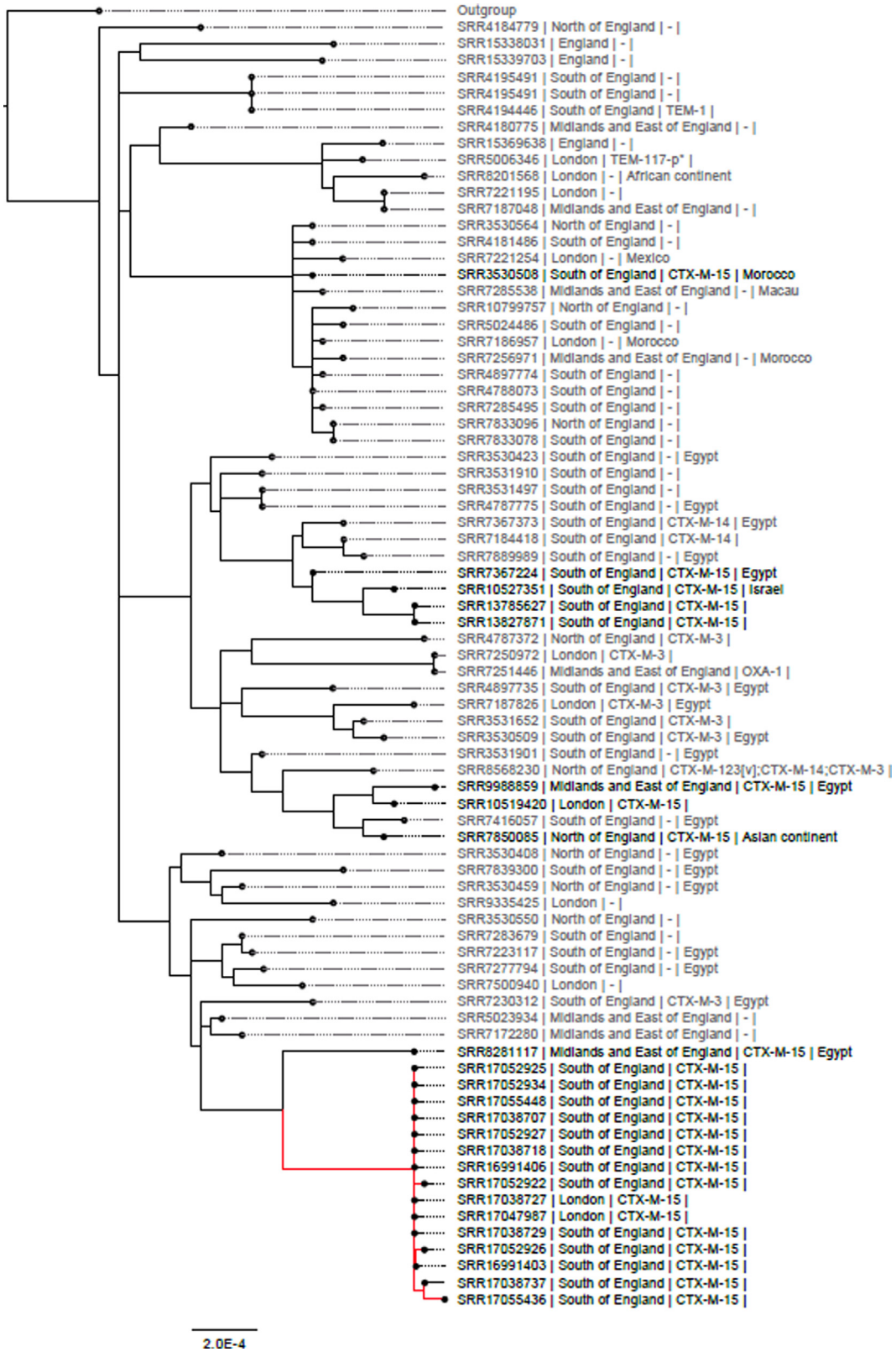

Figure 5. Phylogenetic analysis of the outbreak cluster (highlighted in red) in the context of the most closely related sequences (within a 25 SNP single linkage cluster) in the UKHSA archive. Annotations include the short-read accession number (SRR number), region of residence in the UK, presence of $bla_{\text{CTX-M}}$ variants and travel destination if stated.

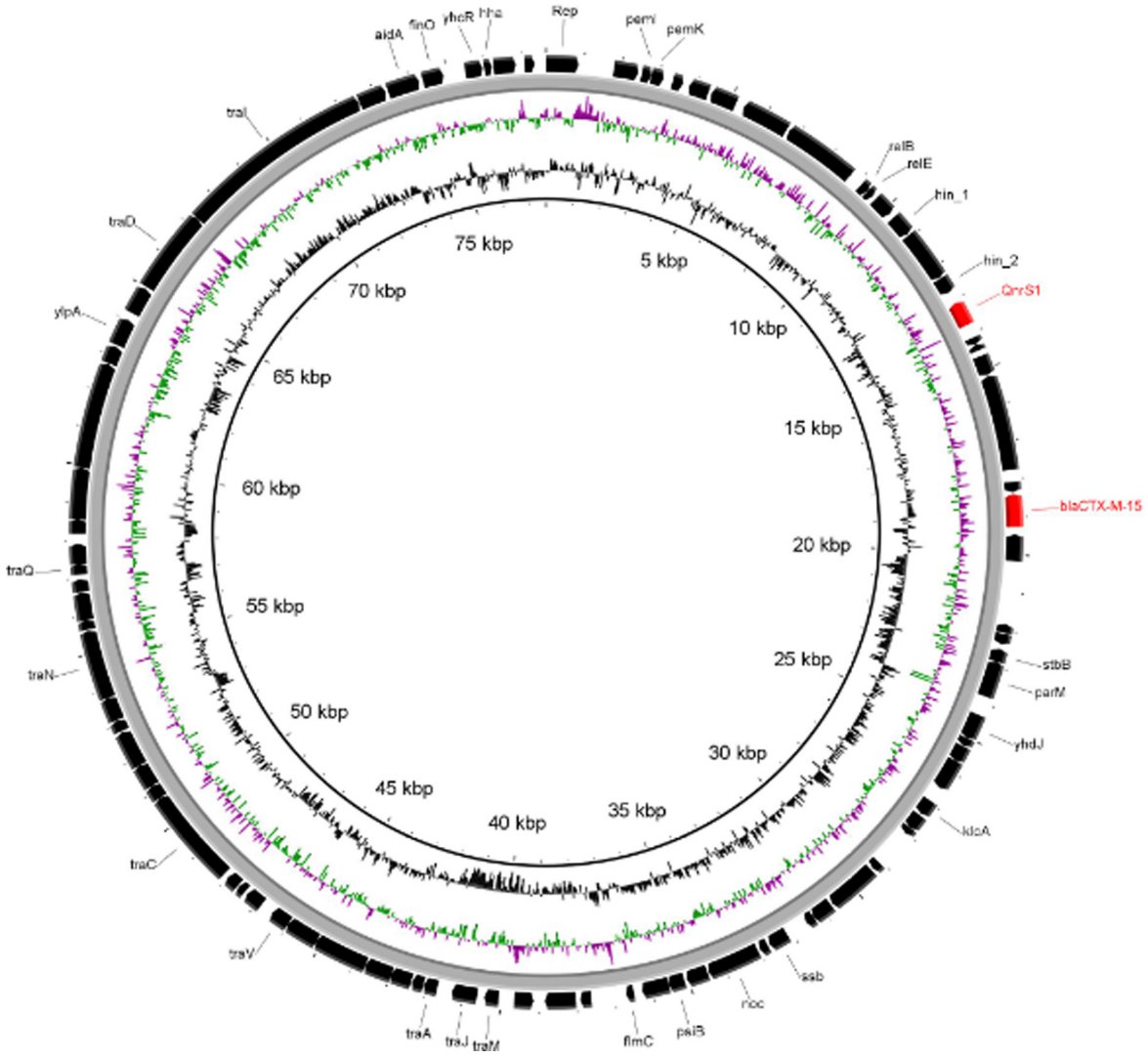

Figure 6. BRIG representation of the 77kbp IncFII plasmid highlighting the location of $bla_{CTX\text{-}M\text{-}15}$ and *qnrS1* in red.

all stakeholders, including local HPTs, EHPs, epidemiologists, microbiologists, and bioinformaticians, as described in this study, will enable us to address the challenges of predicting, detecting, and responding to foodborne threats to public health.

Funding source

CJ and DRG are affiliated to the National Institute for Health Research Health Protection Research Unit (NIHR HPRU) in Gastrointestinal Infections and Genomics and Enabling Data at University of Liverpool and University of Warwick respectively in partnership with the UK Health Security Agency (UKHSA). CJ and DRG are based at UKHSA. The views expressed are those of the author(s) and not necessarily those of the NIHR, the Department of Health and Social Care or the UK Health Security Agency.

Ethical approval

The UKHSA has approval from the Secretary of State for Health and Social Care to collect data for the purpose of diagnosing, recognizing trends, controlling and preventing, and monitoring and managing risks to health. The outbreak investigations described here were completed as a statutory requirement under UKHSA's health protection mandate. As such, ethical approval is not deemed necessary for this work. All cases interviewed, or their legal guardians, consented to taking part in the interview.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors would like to thank all members of the Incident Management Team for their valuable contributions.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jfp.2023.100074.

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